Depressed superoxide radical generation by neutrophils from patients with rheumatoid arthritis and neutropenia: correlation with neutrophil reactive IgG

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SUMMARY Neutrophils of 31 patients with neutropenia and rheumatoid arthritis (RA) have been studied to assess their ability to generate superoxide radicals (O₂⁻) on activation. Seventeen patients had classical Felty’s syndrome and 14 presumed chrysotherapy related neutropenia. Results were compared with those from age and sex matched controls with uncomplicated RA and from normal subjects. Neutrophils from patients with Felty’s syndrome had a significantly reduced ability to generate superoxide radicals when compared with the other three groups. In addition, serum levels of IgG polymorphonuclear leucocyte binding activity (IgG PBA) were also raised in the group with Felty’s syndrome. A statistically significant inverse correlation existed between O₂⁻ generation and IgG PBA. It is concluded that neutrophil reactive IgG may have an important role in both quantitative and qualitative defects in neutrophil function in Felty’s syndrome.

Neutropenia occurring during the course of rheumatoid arthritis (RA) is characteristically seen as a feature of Felty’s syndrome, the pathogenesis of which is complex and multifactorial. It has been shown that patients with this condition may have identifiable marrow suppressor T lymphocytes which suppress granulocyte production. Colony forming units may be depressed as a result of reduced colony stimulating activity, and serum of such patients may contain IgG polymorphonuclear leucocyte binding activity (IgG PBA), and high titres of circulating immune complexes. All these factors collectively or individually may be involved in the development of neutropenia. Furthermore, recent evidence has suggested that polymorphonuclear leucocytes (PMN) in patients with Felty’s syndrome are not only defective in numbers but also in function, with evidence that chemotactic responses, phagocytosis, bactericidal activity, and the ability to generate superoxide radicals upon activation are depressed as compared with patients with uncomplicated rheumatoid arthritis. Factors giving rise to neutropenia may also be incriminated in the functional defects of PMN in Felty’s syndrome. For example, normal PMN incubated in plasma from patients with Felty’s syndrome show a decreased ability to generate superoxide radicals upon activation, which can be directly related to the level of IgG PBA in the test plasma.

In addition to neutropenic patients with Felty’s syndrome, others may be identified who have mild chronic intermittent neutropenia but who do not fulfil the clinical criteria for classical Felty’s syndrome. In some cases this mild neutropenia is attributed to the use of second line therapy without obvious evidence of marrow suppression. This study further reports the relation between serum IgG PBA and the ability of PMN from various neutropenic RA patients to generate superoxide radicals upon activation with chemotactic stimuli.
Patients and methods

Patients
Thirty one patients with classical rheumatoid arthritis and an associated neutropenia have been identified in the Edmonton rheumatic disease unit clinics. Of these patients, seventeen fulfil the clinical criteria for classical Felty’s syndrome as defined by a persistent neutrophil count of less than 1500 PMN/mm³ (1·5 × 10⁹/l) and palpable splenomegaly. A number of these patients had other features seen in Felty’s syndrome, such as recurrent infections, vasculitis, and chronic leg ulcers. Fourteen patients had chronic intermittent and relatively mild neutropenia (1000–2000 PMN/mm³ (1·2 × 10⁹/l)) but without any of the other features of classical Felty’s syndrome. Most of these patients were receiving second line medication consisting of chrysotherapy and d-penicillamine. The other clinical features of these patients have been previously reported.¹¹

The results obtained by using PMN from these subsets of patients were compared with the results obtained from age and sex matched patients with uncomplicated classical rheumatoid arthritis and from a similarly matched group of normal healthy controls.

Methods
Isolation of PMN
Heparinised venous blood was drawn and separated on a Ficoll-Hypaque (Pharmacia (Canada) Ltd, Dorval, PQ) density gradient according to the procedure of Boyum.¹² Lymphocytes and monocytes were removed, and erythrocytes lysed twice with a solution containing 0-83% NH₄Cl, 0-01 M NaHCO₃, and 0-1 mM ethylenediaminetetra-acetic acid (pH 7-4) for five minutes. The PMN were washed twice and resuspended in Hanks' balanced salt solution (pH 7-4) at a final concentration of 5 × 10⁸ PMN/ml. The PMN purity was >95% PMN with >98% viability as assessed by both trypsin blue exclusion and lactic dehydrogenase release assays.

Kinetic assay of O₂ production by PMN
A cytochrome c reduction assay was used to assess generation of O₂ by PMN as previously described.¹³ Two hundred and fifty microlitres of washed cell suspension (0·5 × 10⁶ cells) and 250 µl 0-2 mM ferricytochrome c (Sigma Chemical Co, St Louis, MO) were combined in a 1 cm semimicrocuvette. The cells were activated by the addition of 10 µl 0·05 M fmet-leu-phe (Sigma Chemical Co, St Louis, MO) or 2 µl phorbol 12-myristate 13-acetate (1 × 10⁻⁸ M) (P-L Biochemicals, Milwaukee), and the rate and quantity of O₂ mediated reduction of ferricytochrome c were recorded at 550 nm in a Perkin-Elmer (model 552) spectrophotometer fitted with a thermostated cuvette holder set at 37°C. Assays were performed in duplicate with greater than 95% reproducibility between assays. The specificity of ferricytochrome c reduction was checked in all experiments by the addition of 15 µg superoxide dismutase to a control tube containing the reaction mixture.

IgG PMN binding activity (IgG PBA)
IgG PMN binding activity was measured in frozen serum samples in Seattle in a blinded fashion as previously described.³ Briefly, 5 × 10⁶ PMN in phosphate buffered saline (PBS) are incubated with test serum for 30 minutes at room temperature. After four washes with cold PBS the PMN are recounted, transferred to clean glass tubes, and lysed by three repeated cycles of freezing and thawing. The amount of IgG in the dilute lysed cell suspension is measured by an Fab-anti F(ab')₂ assay. The rabbit antihuman F(ab')₂ antiserum used is rendered specific by immunoabsorption.

Results
IgG PMN binding activity
IgG PBA was measured in 28 normal healthy controls (mean level (SD) 25·9 (5·6) ng IgG/10⁶ PMN). From this data was calculated a normal range of 14·7–37·1 ng IgG/10⁶ PMN. None of the sera tested exceeded a level of 37 ng/10⁶ PMN. Sera from 43 patients with uncomplicated classical rheumatoid arthritis were also examined (mean level of 35·7 ng IgG/10⁶ PMN). Ten (23%) of the samples tested had levels exceeding 37 ng IgG/10⁶ PMN. These levels were only modestly raised, however, in the majority of sera tested. The mean level of IgG PBA in the 14 patients with rheumatoid arthritis and chronic intermittent neutropenia was 46·8 ng IgG/10⁶ PMN. Six (43%) of these patients also had raised but relatively low levels of IgG PBA detected. In contrast, IgG PBA in the sera from 17 patients with Felty’s syndrome showed a mean level of 65·5 ng IgG/10⁶ PMN which was significantly increased compared with both the control group and with the patients with rheumatoid arthritis without Felty’s syndrome (p<0·01). Thirteen of the 17 (76%) patients had raised levels above 37 ng IgG/10⁶ PMN and most of these exceeded twice the upper limit of the normal range. These data confirm the association between increased IgG PBA and Felty’s syndrome, though low levels of this binding activity can also be seen in patients with apparently uncomplicated rheumatoid arthritis (Table 1).
Depressed superoxide radical generation

Superoxide radical generation

The rate and quantity of superoxide radical generation by PMN activated by the chemotactic factor fmet-leu-phe are shown in Table 1. A significant correlation existed between the rate and quantity of O$_2$ generation by cells from individual subjects (p=0.001, r=0.88). Both the rate and total quantity of superoxide radical generation by cells from 28 normal controls, 43 patients with uncomplicated rheumatoid arthritis, and 14 patients with RA and chronic neutropenia produced similar results with no statistically significant difference between the groups. In contrast, the mean rate and quantity of superoxide radical generation by activated cells from patients with Felty’s syndrome was significantly depressed when compared with results for the previous three groups. In particular, cells from seven of the 17 patients had levels below that seen in any sample drawn from the other three control groups. These data confirm and expand previously reported information which indicated that cells from patients with Felty’s syndrome have a decreased ability to generate superoxide radicals upon activation.

Table 1 IgG PMN binding activity and O$_2$ generation

<table>
<thead>
<tr>
<th>Group</th>
<th>No</th>
<th>IgG PMN levels (ng IgG/10$^6$ PMN)</th>
<th>Rate (nmoles/min/10$^6$ PMN)</th>
<th>Quantity (nmoles/10$^6$ PMN)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls</td>
<td>28</td>
<td>25.1 (5-6)</td>
<td>13.5 (5-25)</td>
<td>28.8 (7-4)</td>
</tr>
<tr>
<td>RA</td>
<td>43</td>
<td>35.7 (23-2)</td>
<td>11.8 (4-95)</td>
<td>25.3 (9-3)</td>
</tr>
<tr>
<td>Adrenalopathy</td>
<td>14</td>
<td>46.8 (23-3)</td>
<td>13.7 (5-40)</td>
<td>28.9 (9-4)</td>
</tr>
<tr>
<td>Felty’s syndrome</td>
<td>17</td>
<td>65.7 (28-1)</td>
<td>6.3 (5-45)</td>
<td>14.0 (7.5)</td>
</tr>
</tbody>
</table>

**Fig. 1** Rate of O$_2$ generation vs IgG PBA.

**Fig. 2** Total O$_2$ generation vs IgG PBA.
CORRELATION BETWEEN IgG PBA AND O$_5^-$ GENERATION

In 27 of the 31 RA patients with neutropenia it was possible to draw a correlation between the serum level of IgG PBA and both the rate and quantity of superoxide radical generation by activated cells from these patients. A significant correlation was seen with both the rate and quantity of superoxide radical generation when compared with the level of IgG PBA activity (p<0.003, r=0.56 and p<0.002, r=0.56 respectively) (Figs 1 and 2).

Discussion

This study supports the hypothesis that IgG PBA may be important not only in the development of quantitative but also qualitative defects in neutrophil function in Felty’s syndrome. In this study patients with Felty’s syndrome had increased levels of IgG PBA and an abnormal ability to generate O$_5^-$ radicals. We have also been able to show that a direct correlation exists between depressed superoxide radical generation by activated patient cells and the concomitant level of IgG PBA in homologous serum. These observations may have clinical importance as superoxide radicals and other excited oxygen species have both inflammatory and bactericidal activity.

The mechanism by which chronic mild neutropenia occurs in RA without Felty’s syndrome is at present unknown. As many of these patients are on second line drugs, however, a possible mild inhibitory effect on granulocytopenia has been postulated. Alternatively, these cases may represent mild forms of Felty’s syndrome without expression of the other classical clinical features.

The results seen in our 14 patients with RA and chronic neutropenia were more heterogeneous than those seen in the group with Felty’s syndrome. Raised levels of IgG PBA were found in a few of these patients and were associated in them with a decreased ability of cells to generate superoxide radicals. Most of these patients, however, had normal levels of IgG PBA and superoxide radical generation similar to that of patients with uncomplicated rheumatoid arthritis. It seems likely that these subjects represent a distinct subset of RA patients in whom the mild neutropenia may be drug induced rather than directly related to the disease itself. This possibility is further supported by immunogenetic studies, which indicate that these patients differ from those patients with classical Felty’s syndrome. Thus the prevalence of HLA-DR4 was found to be 93% in the group with Felty’s syndrome but was only 23% in those patients with RA and mild chronic neutropenia alone. Those patients in this group with high levels of IgG PBA and a defect in superoxide radical generation will therefore warrant further evaluation to determine if in the future they develop other features compatible with a clinical diagnosis of Felty’s syndrome.

In conclusion, we have shown that levels of IgG PBA are significantly increased in patients with Felty’s syndrome and that the ability of their neutrophils to generate superoxide radicals is reduced when compared with the ability of the other three groups tested. We have also been able to show that an inverse correlation exists between the level of IgG PBA and the ability of cells to generate superoxide radicals. These data suggest that neutrophil reactive IgG may have a fundamental role not only in the development of neutropenia in Felty’s syndrome but also in inducing functional defects in cells from these patients, potentially predisposing them to an increased risk of pyogenic infection.

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References

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